Cambridge (CIE) AS Biology

Your notes

The Microscope in Cell Studies

Contents

- The Microscope in Cell Studies
- Magnification Calculations
- Eyepiece Graticules & Stage Micrometers
- Resolution & Magnification
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Your notes

Microscope Slide Preparation

Preparing a microscope slide

- **Specimens** can be viewed under a light microscope; this allows some details of cellular material to be observed
- Pre-prepared **permanent slides** can be viewed
  - Such slides are produced by cutting **very thin layers of tissue** which are stained and permanently mounted on a glass slide for repeated use
- Different methods will be used to view different types of specimen, e.g. **temporary slide preparations** can be produced in the school laboratory as described below

Preparing a slide using a liquid specimen

1. Add a few drops containing the liquid sample to a clean slide using a pipette
2. Lower a **coverslip** over the specimen and gently press down to **remove air bubbles**
   - Coverslips protect the microscope lens from liquids and help to prevent drying out

Preparing a microscope slide using a solid specimen

1. Use scissors or a scalpel to cut a small sample of tissue, and peel away or cut a **very thin layer of cells** from the tissue sample
   - The preparation method always needs to ensure that **samples are thin** enough to allow **light to pass through**
2. Place the sample onto a slide
   - A drop of water may be added at this point
3. Apply **iodine stain**
4. Gently lower a coverslip over the specimen and press down to remove any air bubbles

Preparing a microscope slide using onion cells diagram

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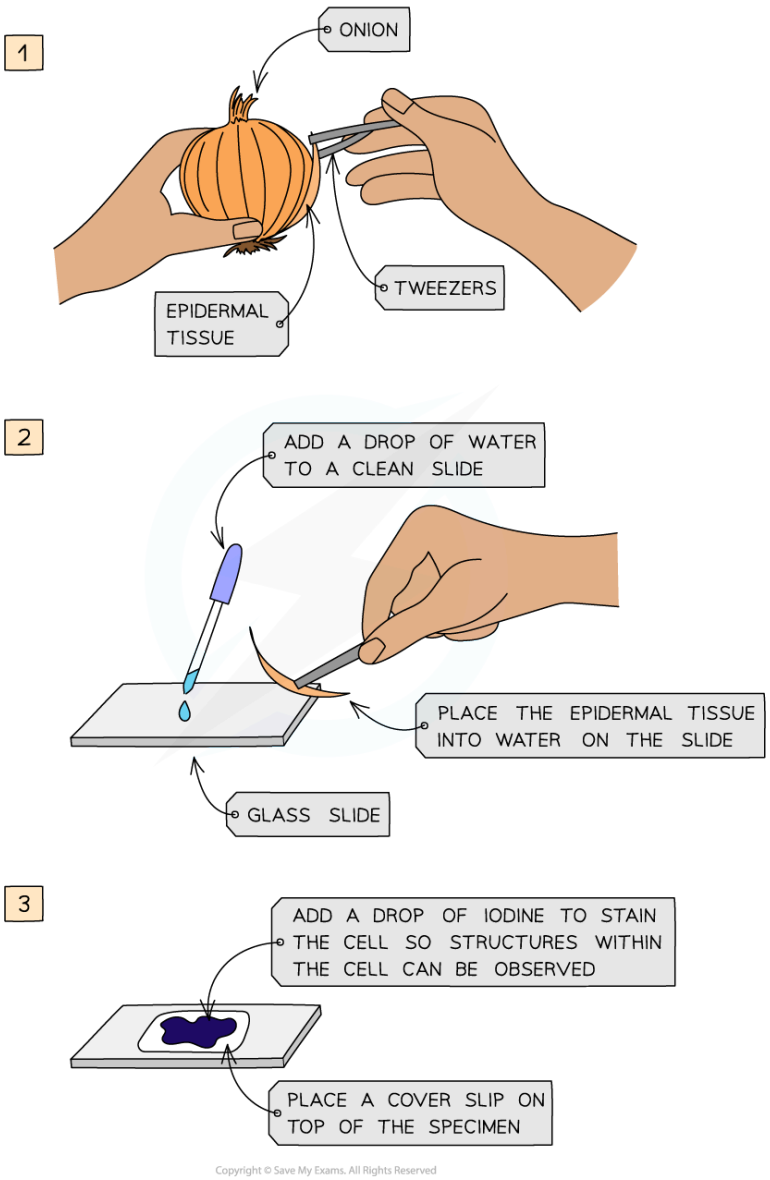
1
ONION
TWEEZERS
EPIDERMAL TISSUE
2
ADD A DROP OF WATER TO A CLEAN SLIDE
PLACE THE EPIDERMAL TISSUE INTO WATER ON THE SLIDE
GLASS SLIDE
3
ADD A DROP OF IODINE TO STAIN THE CELL SO STRUCTURES WITHIN THE CELL CAN BE OBSERVED
PLACE A COVER SLIP ON TOP OF THE SPECIMEN
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Your notes

Tissue from an onion is as a solid specimen, and can be prepared here using iodine stain

Preparing a slide using human cells

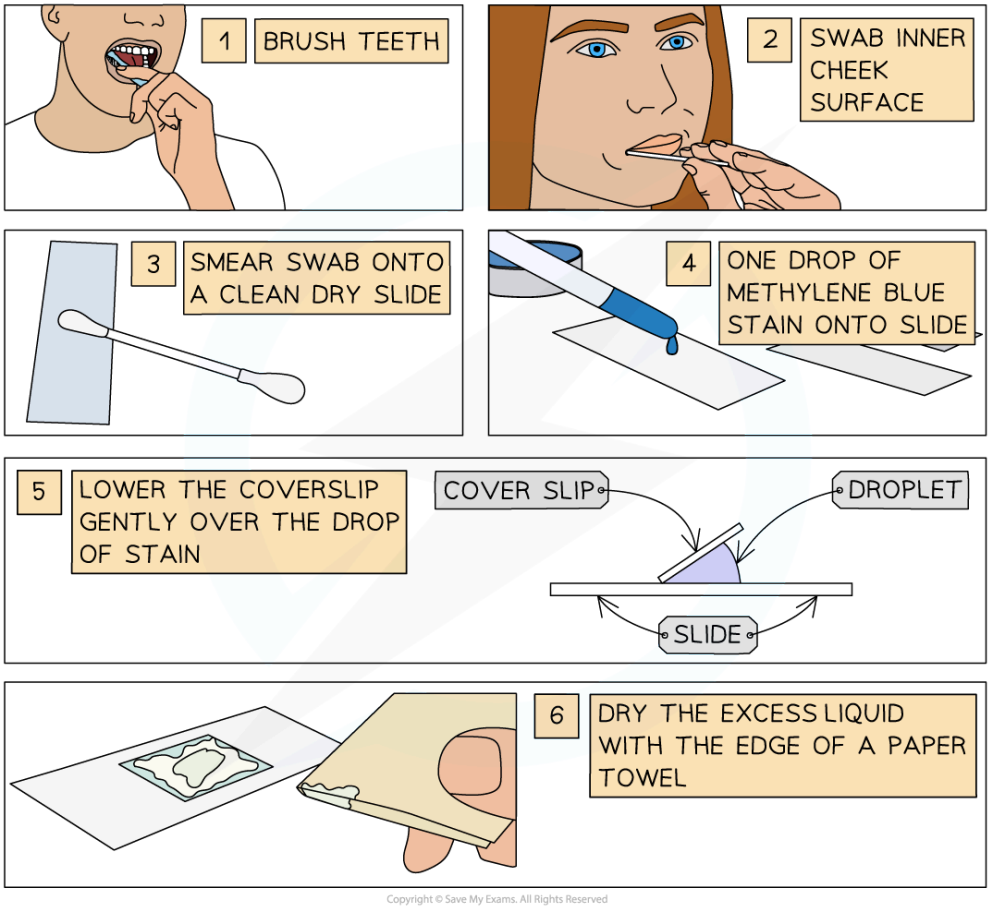
1. Brush teeth thoroughly with normal toothbrush and toothpaste
    - This removes bacteria from teeth so they don't obscure the view of the cheek cells
2. Take a **sterile cotton swab** and gently scrape the inside cheek surface of the mouth for 5–10 seconds
3. Smear the cotton swab on the centre of the microscope slide for 2–3 seconds
4. Add a drop of **methylene blue solution**
    - Methylene blue stains negatively charged molecules in the cell, including DNA and RNA

- This causes the nucleus and mitochondria to appear darker than their surroundings

5. Place a coverslip on top
    - Lay the coverslip down at one edge and then **gently lower** the other edge until it is flat
    - This reduces bubble formation under the coverslip
6. Absorb any excess solution by allowing a paper towel to touch one side of the coverslip

Your notes

Preparing a microscope slide using cheek cells diagram



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Cheek cells can be stained using methylene blue

Staining specimens

- The cytoplasm and other cell structures may be transparent or difficult to distinguish; **stains** allow them to be viewed clearly under a light microscope
- As with the type of preparation required, the type of stain used is dependent on the specimen being viewed

Common microscope stains & uses table

Stain	Uses

Iodine	Stains starch blue-black, and colours nuclei and plant cell walls pale yellow
Crystal violet	Stains cell walls purple
Methylene blue	Stains animal cell nuclei dark blue
Congo red	Is not taken up by cells and stains the background red, so providing contrast with any cells present

Your notes

Drawing Cells

- To record the observations seen under a microscope, a labelled **biological drawing** is often made
- Biological drawings are **line drawings** which show specific features that have been observed when the specimen was viewed
- There are a number of rules/conventions that are followed when making a biological drawing
  - The drawing must have a **title**
  - The **magnification** under which the observations shown by the drawing are made should be recorded if possible
    - A scale bar may be used
  - A **sharp pencil** should be used
  - Drawings should be on plain white paper
  - Lines should be **clear**, **single lines** without sketching
  - **No shading** should be used
  - The drawing should take up as much of the space on the page as possible
  - Well-defined structures should be drawn
  - **Only visible structures** should be drawn, and the drawing should **look like the specimen**
  - The drawing should be made with **proper proportions**
  - Structures should be **clearly labelled** with label lines that:
    - Do not cross
    - Do not have arrowheads
    - **Connect directly** to the part of the drawing being labelled
    - Are on one side of the drawing

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- Are drawn with a **ruler**
- Drawings of cells are typically made when visualizing cells at a higher magnification power, whereas plan drawings are typically made of tissues viewed under lower magnifications (individual cells are never drawn in a plan diagram)

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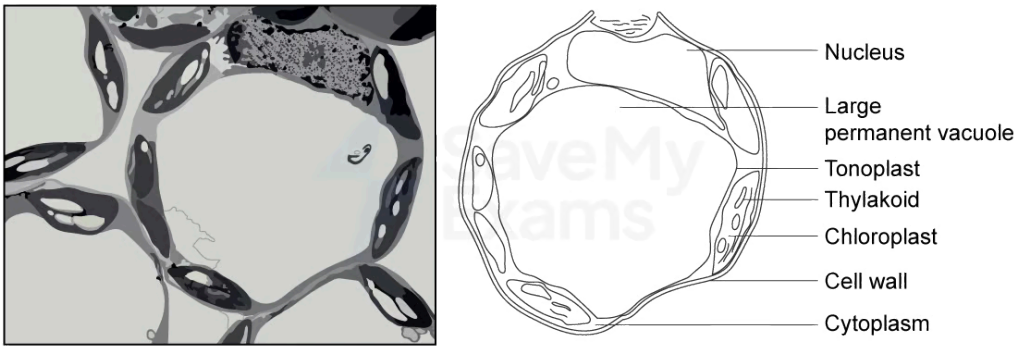
Plant cell biological drawing



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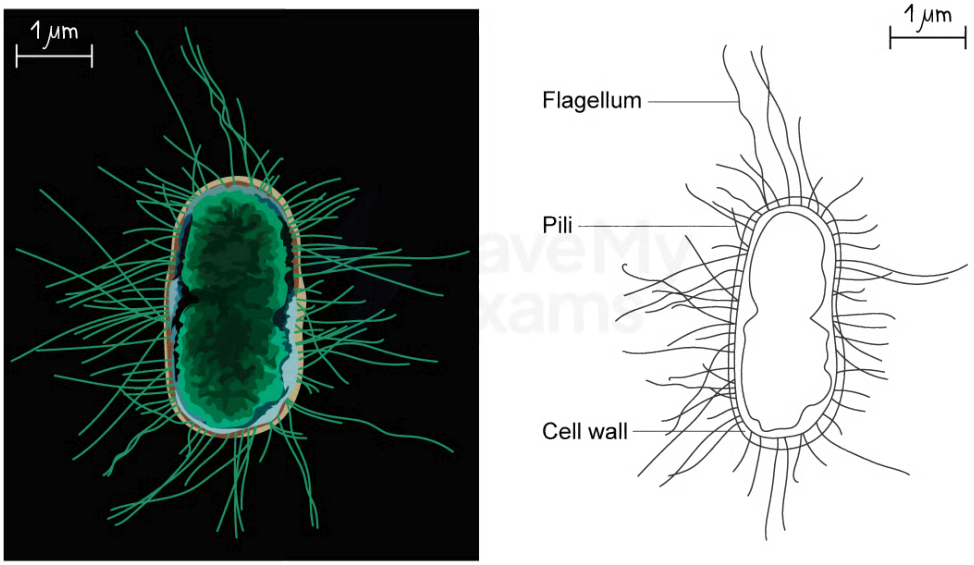
Bacterial cell biological drawings



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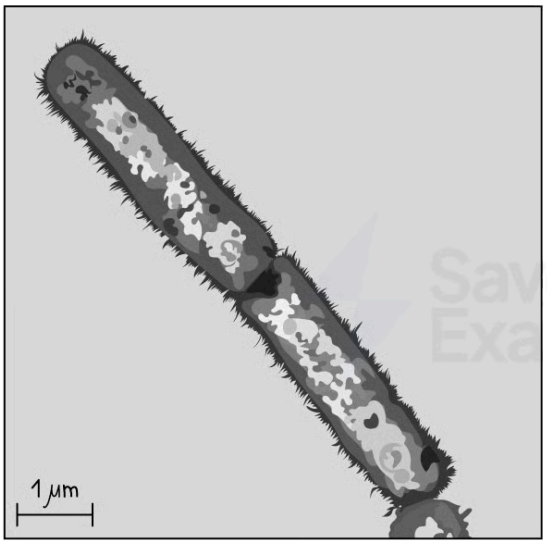

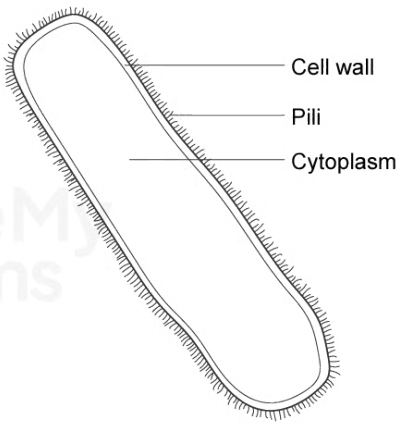

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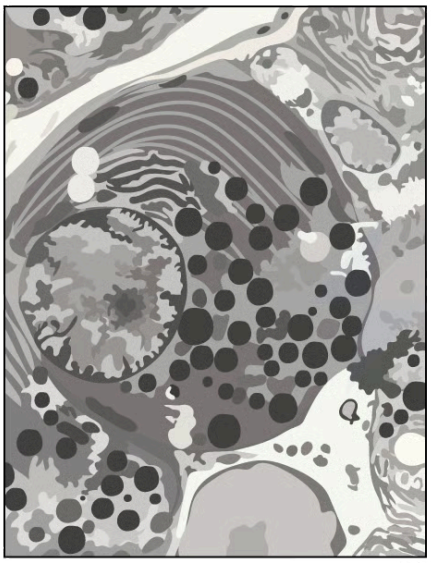
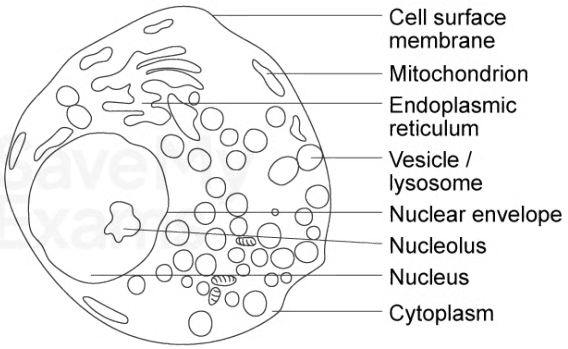
Animal cell drawing





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Examiner Tips and Tricks

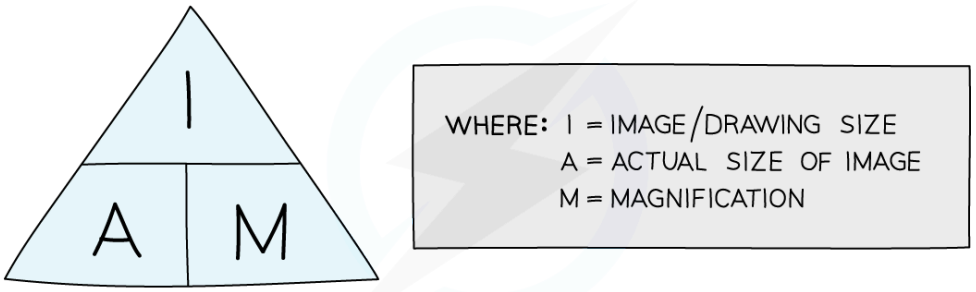
When producing a biological drawing, it is vital that you only ever draw what you see and not what you think you should see!

Your notes

Your notes

Magnification Calculations

- **Magnification** is the **number of times** that a real-life specimen has been **enlarged** to give a larger view/image
  - E.g. a magnification of x100 means that a specimen has been enlarged 100 times to give the image shown
- The **magnification** (*M*) of an object can be calculated if both the size of the image (*I*), and the actual size of the specimen (*A*), is known



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An equation triangle allows the magnification equation to be easily rearranged

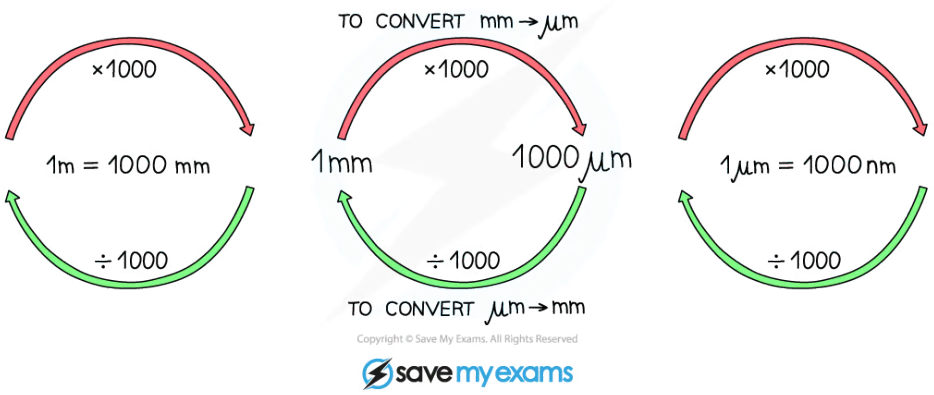
- When carrying out calculations relating to magnification, the following steps should be followed:
  1. Rearrange the equation
     - $A = I \div M$
     - $M = I \div A$
     - $I = A \times M$
  2. Read and measure the relevant values from the question stem and/or any images provided
  3. Convert any units
  4. Substitute numbers into the rearranged equation
  5. Consider whether this value makes sense in the context provided

Converting units during magnification calculations

- Cellular structures are usually measured in either **micrometers** (μm) or **nanometers** (nm), while any measurements carried out in an exam with a ruler are likely to be in **millimeters** (mm)

- There are 1000 µm in a mm
- There are 1000 nm in a µm

- When doing calculations all measurements must be expressed using the **same units**
  - It is best to use the **smallest unit** of measurement shown in the question
  - Note that **magnification does not have units**
- To convert units, multiply or divide depending on whether the units are **increasing or decreasing**
  - Multiply when converting from a larger unit to a smaller unit
  - Divide when converting a smaller unit to a larger unit

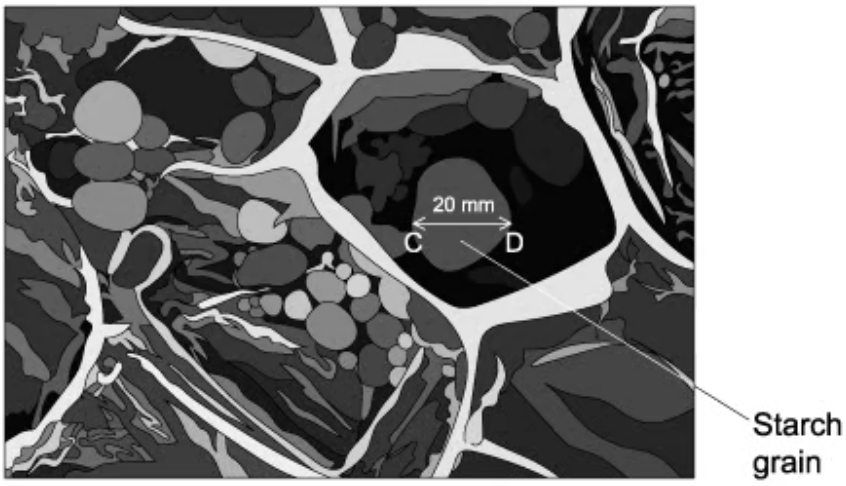


Units can be multiplied or divided by a factor of 1000 when converting between mm and µm

Worked Example

A starch grain inside a plant cell was viewed under a microscope at a magnification of x850. The image of the starch grain captured using the microscope is shown below.

Your notes



Your notes

Calculate the actual diameter of the starch grain between points C-D in the image.

Give your answer in µm.

Step 1: Rearrange the equation

We have been asked to calculate actual size, *A*, so the new equation should be

Actual size = image size ÷ magnification

Step 2: Read and measure relevant values

We need to know the image size and the magnification

The image size has been given in the image as 20 mm

The magnification has been included in the question stem and is x850

Step 3: Convert any units

The actual diameter of a structure inside a cell would normally be measured in µm

The image size has been given in mm, so we need to convert this to µm

1 mm = 1000 µm, so we multiply mm by 1000 to give the value in µm

20 x 1000 = 20 000 µm

Step 4: Substitute number into the equation

Actual size = 20 000 ÷ 850

= 23.53 µm

Step 5: Consider whether the value makes sense

Plant cells measure between 10–100 µm, and the starch grain takes up a large proportion of the cell in the image. A diameter of around 23 µm could therefore be right for this starch grain

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If we had forgotten to convert the units and come up with a value of 0.024 µm, then this step would show us that this is probably too small and that we must have therefore made a mistake somewhere

Your notes

Examiner Tips and Tricks

Magnification calculations will usually involve measuring images on a page, so you must have a **ruler** with you in an exam!

Be aware that the unit conversions given above only work if the **initial measurement is taken in mm, not in cm**. Any measurements taken in cm will need to be converted into mm before multiplying by 1000 to give µm.

Some exam questions may expect you to **use a scale bar** to calculate the magnification of an image. Remember that you can use a scale bar to find both actual size (written on the scale bar) and image size (the measured length of the bar), so these values can be used to calculate magnification using the equation M = I ÷ A

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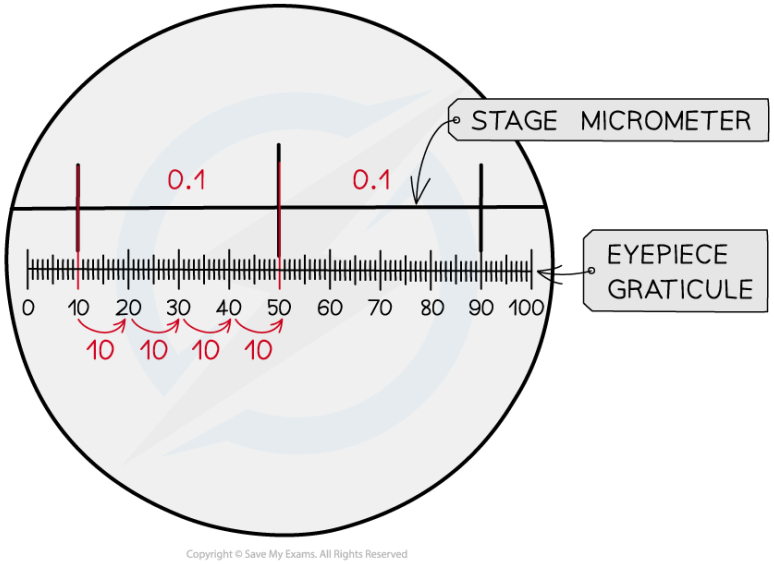
Eyepiece Graticules & Stage Micrometers

- An **eyepiece graticule** and **stage micrometer** are used to measure the size of an object when viewed under a microscope
- The eyepiece graticule is an engraved ruler that is visible when looking through the eyepiece of a microscope
  - Eyepiece graticules are often divided into 100 smaller divisions known as **graticule divisions**, or **eyepiece units**
- The values of the divisions in an eyepiece graticule **vary depending on the magnification used,** so the graticule needs to be **calibrated** every time an object is viewed
- The calibration is done using a stage micrometer; this is a slide that contains a tiny ruler with an accurate known scale
  - Stage micrometer rulers can vary, but often have larger divisions of 0.1 mm (100 µm) and smaller divisions of 0.01 mm (10 µm)

Calibrating the eyepiece graticule



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- In the diagram, two stage micrometer divisions of 0.1 mm, or 100 µm, are visible
- Each 100 µm division is equal to 40 eyepiece graticule divisions
  - 40 graticule divisions = 100 µm

1 graticule division = number of µm ÷ number of graticule divisions

- 1 graticule division = 100 ÷ 40 = 2.5 µm; this is the **magnification factor**

Calculating the size of a specimen

- The calibrated eyepiece graticule can be used to measure the length of an object
- The number of graticule divisions covered by an object need to be multiplied by the magnification factor:

graticule divisions covered by object x magnification factor = length of object (μm)

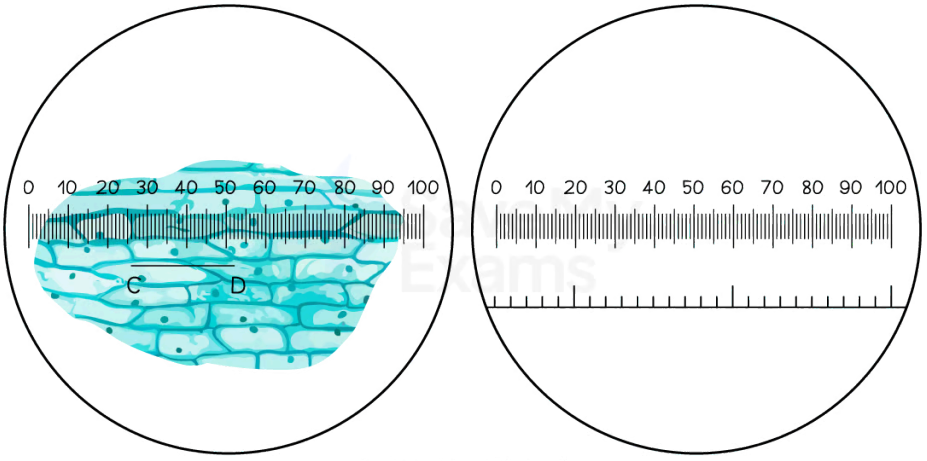
Your notes

Worked Example

A student viewed some onion cells under a microscope.

The image below shows the cells with an eyepiece graticule (left) and the eyepiece graticule alongside a stage micrometer (right).

Note that each large division on the stage micrometer here is 100 μm, and each small division is 10 μm.



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Use the stage micrometer to calibrate the eyepiece graticule and calculate the actual length of the cell labelled C-D in the image.

Step 1: Calculate the size of each eyepiece division

There are 40 graticule divisions per large micrometer division, or per 100 μm

1 graticule division = no. of μm ÷ no. of graticule divisions

= 100 ÷ 40

= 2.5 μm

This value can now be used as a **magnification factor**

Step 2: Calculate the length of the cell

Specimen size = no. of graticule divisions x magnification factor

The cell closest to the ruler covers 27 graticule divisions

= 27 x 2.5

= 67.5 µm

Step 3: Consider whether this answer makes sense in context

Plant cells usually measure between 10–100 µm, so a result of 67.5 µm sounds sensible in this context.

Examiner Tips and Tricks

The calculations involving stage micrometers and eyepiece graticules are often seen in exam questions, so make sure that you are comfortable with how to calibrate the graticule and calculate the length of an object on the slide.

Note that both of the examples given above are carried out at the same magnification, so the magnification factor calculated during calibration is 2.5 in both cases. This may not always be the same in an exam.

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Your notes

Resolution & Magnification

Magnification

- Magnification is the number of times that a real-life specimen has been enlarged to give a larger view/image
  - E.g. a magnification of x100 means that a specimen has been enlarged 100 times to give the image shown
- A **light microscope** has two types of lens which allow it to achieve different levels of magnification:
  - An **eyepiece lens,** which often has a magnification of x10
  - A series of **objective lenses**, each with a different magnification, e.g. x4, x10, x40 and x100
- To calculate the **total magnification** of a specimen being viewed, the magnification of the **eyepiece** lens and the **objective** lens are **multiplied** together:

total magnification = eyepiece lens magnification x objective lens magnification

Resolution

- The **resolution** of a microscope is its ability to **distinguish two separate points on an image as separate objects**; this determines the ability of a microscope to show detail
  - If resolution is too low then two separate objects will be observed as one point, and an image will appear blurry, or an object will not be visible at all
  - The resolution of a microscope **limits the magnification that it can usefully achieve**; there is no point in increasing the magnification to a higher level if the resolution is poor
- The resolution of a light microscope is **limited by the wavelength of light**
  - Visible light falls within a set range of light wavelengths; 400–700 nm
  - The resolution of a light microscope **cannot be smaller than half the wavelength** of visible light
    - The shortest wavelength of visible light is 400 nm, so the maximum resolution of a light microscope is **200 nm**
  - E.g. the structure of a phospholipid bilayer cannot be observed under a light microscope due to low resolution:
    - The width of the phospholipid bilayer is about 10 nm
    - The maximum resolution of a light microscope is 200 nm, so any points that are separated by a distance of less than 200 nm, such as the 10 nm phospholipid

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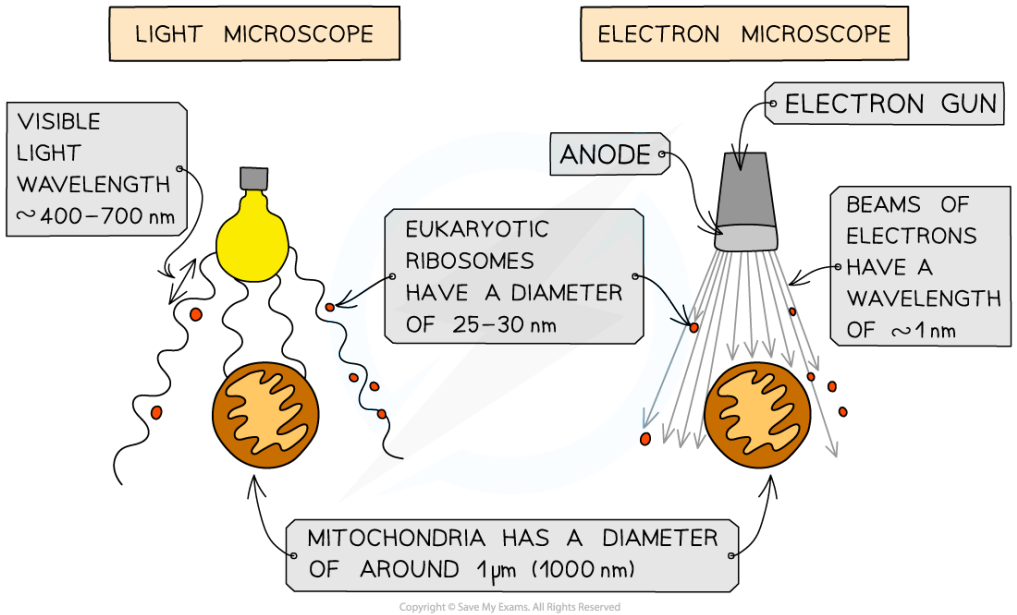
bilayer, cannot be resolved by a light microscope and therefore will not be distinguishable as separate objects

- **Electron microscopes** have a much higher resolution, and therefore magnification, than light microscopes as **electrons have a much smaller wavelength** than visible light
  - Electron microscopes can achieve a resolution of **0.5 nm**

Resolution of light and electron microscopes diagram


LIGHT MICROSCOPE
ELECTRON MICROSCOPE
VISIBLE LIGHT WAVELENGTH ~400–700 nm
ELECTRON GUN
ANODE
EUKARYOTIC RIBOSOMES HAVE A DIAMETER OF 25–30 nm
BEAMS OF ELECTRONS HAVE A WAVELENGTH OF ~1 nm
MITOCHONDRIA HAS A DIAMETER OF AROUND 1 μm (1000 nm)
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The resolving power of electron microscopes is much greater than that of light microscopes due to the smaller wavelength of electrons in comparison to visible light

Comparison of light and electron microscopes

- **Light microscopes** are used for specimens larger than **200 nm**
  - Light microscopes shine **light** through the specimen
  - The specimens can be **living**, and therefore can be moving, **or dead**
  - Light microscopes are useful for looking at **whole cells**, small plant and animal **organisms**, and **tissues within organs** such as in leaves or skin
- **Electron microscopes**, both scanning and transmission, are used for specimens larger than **0.5 nm**
  - Electron microscopes fire a **beam of electrons** at the specimen
    - Transmission electron microscopes (TEM) fire electrons *through* a specimen
    - Scanning electron microscopes (SEM) bounce electrons *off the surface* of a specimen
  - The electrons are picked up by an electromagnetic lens which then shows the image

Your notes

- Electron microscopy requires the specimen to be **dead**, meaning that they can only be used to capture a snapshot in time, and not active life processes as they occur
- Electron microscopes are useful for looking at **organelles, viruses** and **DNA** as well as looking at whole cells in more detail

Your notes

Comparing light & electron microscopes table

Electron microscope	Light microscope
Large machines that are permanently installed in laboratories	Small and portable
Need to create a vacuum for electrons to travel through	No vacuum required
Specimen preparation is complex	Specimen preparation can be simple
Maximum magnification of x500 000	Maximum magnification of x2000
Maximum resolution of 0.5 nm	Maximum resolution of 200 nm
Specimens are always dead	Specimens can be living or dead

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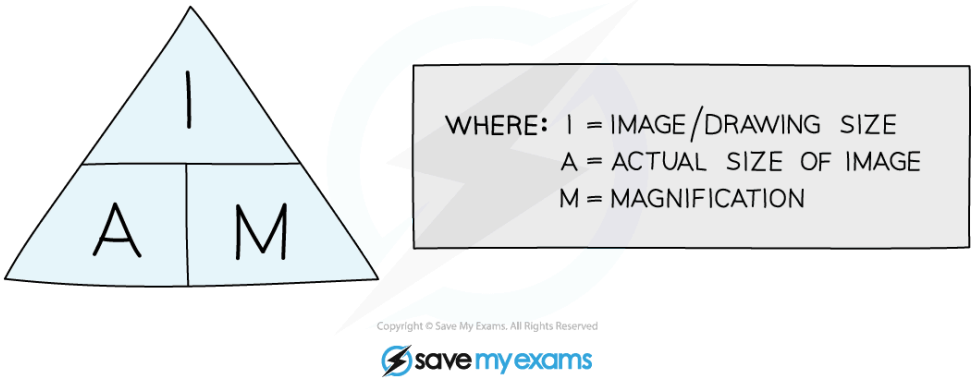
Calculating Actual Size

- When using microscopes to study biological specimens, it is possible to calculate the **actual size** of organisms, cells, and parts of cells
- The actual size of specimens can be calculated using the **magnification** and the **measured size of an image** as follows:

  Actual size = image size ÷ magnification

  - Magnification is sometimes provided in an exam question stem
  - Magnification can be calculated from the **scale bar** of an image
  - Sometimes magnification is calculated from information about the magnification of the eyepiece lens and the objective lens
- Remember that the equation above is part of the equation triangle from calculating magnification



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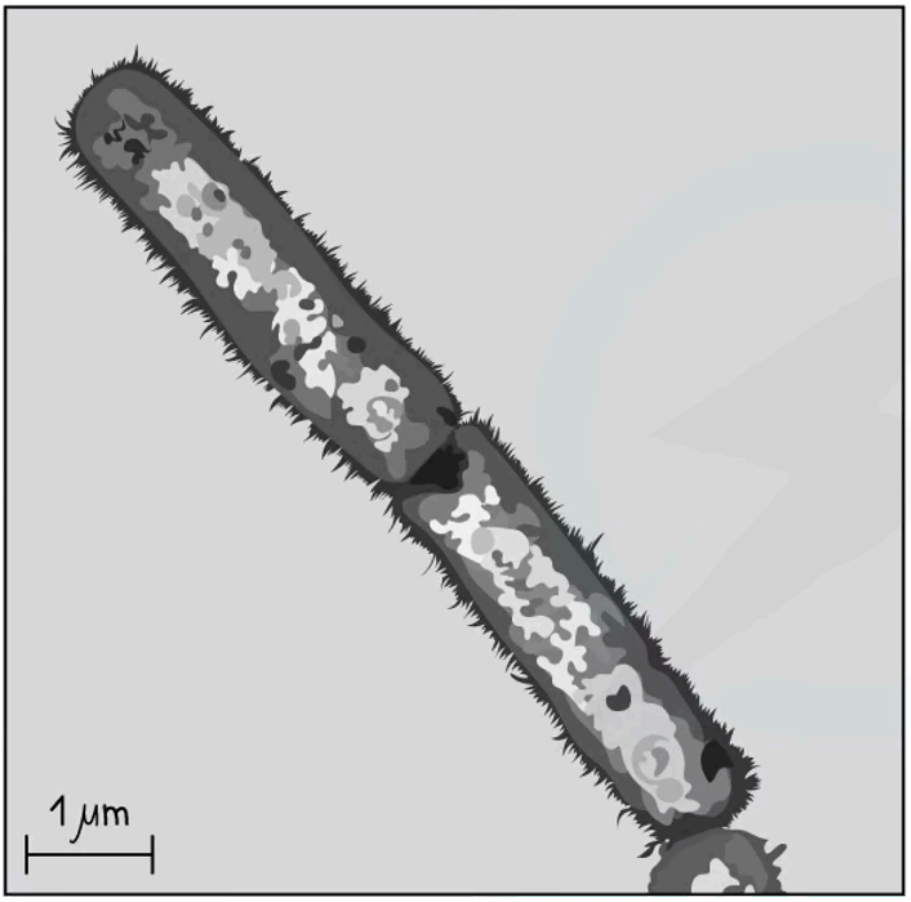
Worked Example

Using a scale bar to calculate actual size

A lab technician observed bacterial cells with an electron microscope, and produced the image below.

The scale bar measures 2 cm in length, and the length of the technician's image of one bacterial cell measures 7.6 cm.



Use the information provided to calculate the actual length of a bacterial cell in the image.

Step 1: Use the scale bar to calculate the magnification of the image

The equation triangle for magnification tells us that:

Magnification = image size ÷ actual size

The scale bar measures 2 cm = 20 mm = 20 000 μm

The scale bar represents an actual size of 1 μm

Magnification = 20 000 ÷ 1

= 20 000

Step 2: Substitute values into the equation for actual size

Actual size = image size ÷ magnification

The question stem tells us that one cell = 7.6 cm = 76 mm = 76 000 μm

Magnification is ×20 000

Actual size = 76 000 ÷ 20 000

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= 3.8

Therefore, the actual length of a bacterial cell in this image is **3.8 µm**

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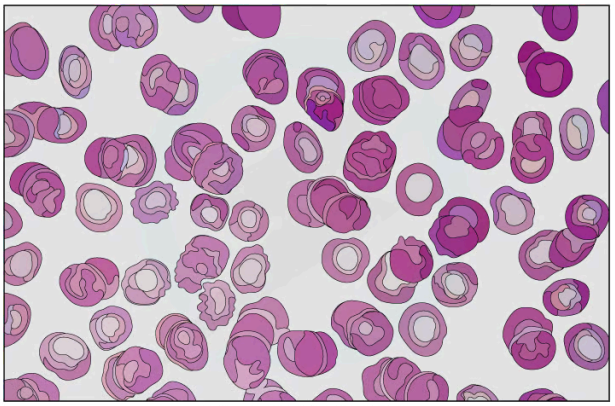
Worked Example

Using lens magnification to calculate actual size

A scientist looked at a sample of red blood cells under a light microscope.

The eyepiece lens of the microscope had a magnification of ×10 and the objective lens had a magnification of ×40.

The scientist produced a photomicrograph of the blood cells, shown below, in which the red blood cells have an average diameter of 3 mm when measured using a ruler.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Calculate the average diameter of the red blood cells in the sample. Give your answer in micrometres.

Step 1: Calculate the total magnification of the specimen

total magnification = eyepiece lens magnification × objective lens magnification

10 × 40 = 400

Magnification = ×400

Step 2: Convert the image size into µm

1 mm = 1000 µm

3 × 1000 = 3000

Image size = 3000 µm

Step 3: Substitute values into equation for actual size

Actual size = image size ÷ magnification

Actual size = 3000 ÷ 400

= 7.5

Therefore, the average size of a red blood cell in this sample is **7.5 µm**

Your notes

Examiner Tips and Tricks

Note that you would be expected to use your own ruler to provide image size measurements in actual size calculations.

Don't forget to convert units in calculations like this!

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